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## SUMMARY

The toxicity of nine different pesticides or degradation products to the groundwater copepod *Parastenocaris germanica* was investigated in 96 hours exposure experiments with immobility and mortality as end points. Test compounds were selected for their potential hazard for groundwater metazoans. The experiments did not always result in distinct dose-response relationships or give acceptable reproducible results. This was because of variance in observations probably associated with the experimental design (small volume and amounts of animals) or heterogeneity of biological material (field population origin).

The most toxic chemicals to *P. germanica* appeared to be Fenpropathrin ( $EC_{50}$ : 0.006  $\mu\text{g/l}$ ) and Cypermethrin ( $EC_{50}$ : 0.02  $\mu\text{g/l}$ ), followed by Thiram ( $EC_{50}$ : 2.3  $\mu\text{g/l}$ ); moderately toxic were Aldicarb-sulfoxide ( $EC_{50}$ : <10  $\mu\text{g/l}$ ), Aldicarb-sulfon ( $EC_{50}$ : <180  $\mu\text{g/l}$ ), MITC ( $EC_{50}$ : 32  $\mu\text{g/l}$ ), and Ethoprofos ( $EC_{50}$ : 450  $\mu\text{g/l}$ ); and the least toxic was ETU ( $EC_{50}$ : 2330  $\mu\text{g/l}$ ). Results of an experiment with Propoxur were invalid because of 100% mortality in the solvent control.

Close similarity in ecotoxicological profiles of *P. germanica* and *Daphnia* sp. for the investigated compounds seems to exist. This suggests that data from aquatic ecotoxicology can be used in the preliminary ecological hazard assessment of pesticides in groundwater.

## SAMENVATTING

De toxiciteit van negen verschillende bestrijdingsmiddelen of afbraakproducten voor de grondwater copepode *Parstenocaris germanica* is onderzocht. De onderzochte stoffen zijn geselecteerd vanwege hun potentieel gevaar voor grondwater bewonende metazoën. Niet alle experimenten lieten een duidelijke dosis-respons relatie zien of leverden goed reproduceerbare resultaten op. Dit werd veroorzaakt door een grote variatie in de waarnemingen mogelijk veroorzaakt door het technisch ontwerp van de experimenten (kleine volumina en aantallen test organismen) of door heterogeniteit van het biologisch materiaal (afkomstig van een veldpopulatie).

Het meest toxisch voor *P. germanica* bleken Fenprothrin ( $EC_{50}$ : 0.006  $\mu\text{g/l}$ ) en Cypermethrin ( $EC_{50}$ : 0.02  $\mu\text{g/l}$ ) te zijn, gevolgd door Thiram ( $EC_{50}$ : 2.3  $\mu\text{g/l}$ ); matig toxisch waren Aldicarb-sulfoxide ( $EC_{50}$ : <10  $\mu\text{g/l}$ ), Aldicarb-sulfon ( $EC_{50}$ : <180  $\mu\text{g/l}$ ), MITC ( $EC_{50}$ : 32  $\mu\text{g/l}$ ), en Ethoprosfos ( $EC_{50}$ : 450  $\mu\text{g/l}$ ); het minst toxisch was ETU ( $EC_{50}$ : 2330  $\mu\text{g/l}$ ). De resultaten van het experiment met Propoxur waren invalide vanwege 100% sterfte in de controle met oplosmiddel.

Voor de onderzochte stoffen lijkt er een grote overeenkomst te zijn in het ecotoxicologisch profiel van *P. germanica* en *Daphnia* sp. Dit suggereert dat in de voorlopige ecologische risico analyse van bestrijdingsmiddelen in grondwater gegevens uit de aquatische ecotoxicologie kunnen worden gebruikt.

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## 1. INTRODUCTION

Within the framework of the soil ecotoxicology subprogramme of the Netherlands Integrated Soil Research Programme, the ecotoxicology of groundwater inhabiting organisms was studied. Part of this research resulted in an operational method for testing the acute toxicity of chemicals to the harpacticoid groundwater copepod *Parastenocaris germanica* (Notenboom & Boessenkool, 1992; Notenboom *et al.*, 1993). The design of this method makes it appropriate for investigating pesticide toxicity. In this study the test system is applied to investigate the toxicity of several pesticides and some of their metabolites to *P. germanica*.

Selection of compounds tested was based on their potential to cause adverse effects on groundwater biota. Preliminary screening was done by combining data on expected or measured environmental concentrations and aquatic toxicity. Results of the present investigation are used to evaluate the technical aspects of the test method. Moreover, it enables an ecotoxicological contribution to the analysis of environmental risks of groundwater pesticides.

## 2. SELECTION OF PESTICIDES

Selection of investigated pesticides was based on a preliminary screening. This was done by comparing groundwater concentration estimates with acute toxicity data of epigeal crustaceans (Notenboom *et al.*, 1992). Groundwater concentration estimates are derived from calculations with the pesticide leaching model PESTLA (Boesten & Van der Linden, 1991), or based on available measurements of pesticides in Dutch ground water (Hopman *et al.*, 1990; Lagas *et al.*, 1991). Relative risks of groundwater pesticides are ranked according to their PEC/NEC or MEC/NEC ratio's<sup>1</sup>.

Based on their species number, phylogenetic diversity, and often their biomass, crustaceans may be regarded as the most important taxon of groundwater metazoans (Botosaneanu, 1986). Therefore, data from epigeal crustaceans are considered relevant for deriving preliminary risk levels. Moreover, taxonomically close species often show similar ecotoxicological profiles (Vaal *et al.* 1994). NEC values are calculated by:  $\alpha \cdot L(E)C_{50}$ .  $EC_{50}$  values prevailed and when adequate data from more species was available, the geometric mean of  $EC_{50}$  values was taken. For  $\alpha$  the values 1, 0.1, or 0.01 are taken. Values for  $\alpha < 1$  seem to be justified because safe levels for ecosystems are lower than a single  $L(E)C_{50}$  value, data are obtained in short-term experiments while long-term exposure prevails in ground water, and the large number of uncertainties which had to be considered.

PEC and  $\alpha \cdot L(E)C_{50}$  values are extracted from environmental information of 220 pesticides reevaluated during the first phase of a catch-up operation of old pesticides (Canton *et al.*, 1991). For 139 of these substances, appropriate information exists that enable calculation of PEC values with the PESTLA model, resulting in 83 pesticides with PEC values  $\geq 0.1 \mu\text{g/l}$  (Notenboom *et al.*, 1992). For other pesticides, PESTLA gives the mean ( $PEC_{(\text{mean})}$ ) and maximal ( $PEC_{(\text{max})}$ ) concentrations of the pesticides in the uppermost ground water, both values are used for risk calculations. Pesticides exceeding risk values with different values for  $\alpha$  and both PEC values are given in Table 1.

Table 2 gives the pesticides and their degradation products encountered in Dutch ground water in concentrations  $\geq 0.1 \mu\text{g/l}$ . This picture is probably incomplete since the information was derived

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<sup>1</sup> PEC = predicted environmental concentration; MEC = measured environmental concentration; NEC = no effect concentration.

**Table 1.** Pesticides exceeding the risk value  $\alpha \cdot L(E)C_{50}$ , with for  $\alpha$  1, 0.1, and 0.01. Predicted environmental concentrations based on mean and worst case conditions of PESTLA. Pesticides mentioned only at the risk category with highest  $\alpha$ -value.

|                       | $\alpha=1$                                                                                                                                                 | $\alpha=0.1$                                         | $\alpha=0.01$                                                                                                               |
|-----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|
| PEC <sub>(mean)</sub> | 1,3-dichloropropene                                                                                                                                        | propoxur<br>thiram<br>trichlorphon                   |                                                                                                                             |
| PEC <sub>(max)</sub>  | 1,3-dichloropropene<br>cypermethrin<br>fenpropathrin<br>pendimethalin<br>pirimicarb<br>pirimiphos-methyl<br>propoxur<br>terbufos<br>thiram<br>trichlorphon | cyfluthrin<br>ethoprophos<br>pyrazofos<br>triazophos | atrazine<br>deltamethrin<br>dinoseb<br>fenaminosulf<br>fenithrothion<br>fonophos<br>furalaxyl<br>oxamyl<br>parathion-methyl |

**Table 2.** Review of pesticides and residues detected in Dutch ground water (0.5-10 m below surface) with the range of their concentrations. Metabolites and admixtures are underlined. Based on Hopman *et al.* (1990) and Lagas *et al.* (1991).

|                                    |                                    |
|------------------------------------|------------------------------------|
| <b>0.1-1 µg/l</b>                  | <b>10-100 µg/l</b>                 |
| alachlor                           | <u>aldicarb-sulfon</u>             |
| amitrol                            | bromacil                           |
| <u>anthranilacidisopropylamide</u> | 1,3-dichloropropene                |
| carbendazim                        | <u>ethyleenthioneum</u> (ETU)      |
| cyanazine                          | <u>methylisothiocyanate</u> (MITC) |
| diuron                             | <u>1,2,3-trichloropropane</u>      |
| lindane ( $\gamma$ -HCH)           |                                    |
| metalaxyl                          |                                    |
| metalochlor                        |                                    |
| metamitron                         |                                    |
| oxamyl                             |                                    |
| simazine                           |                                    |

Table 2 (cont.)

| 1-10 µg/l                | >100 µg/l                          |
|--------------------------|------------------------------------|
| atrazine                 | <u>1,2-dichloropropane</u>         |
| <u>de-atrazine</u>       | <u>2,6-dichlorobenzamide (BAM)</u> |
| <u>dip-atrazine</u>      |                                    |
| bentazone                |                                    |
| <u>chlorallylalcohol</u> |                                    |
| dikegulac-sodium         |                                    |
| dinoseb                  |                                    |
| ethoprosfos              |                                    |
| mecoprop-p               |                                    |
| terbutryn                |                                    |

from limited monitoring programs, and detection methods are not available for all compounds (including metabolites) which could be expected. The highest concentrations reported are taken as  $MEC_{(max)}$  and used for preliminary risk evaluation (worst case scenario). This was not possible for all pesticides recorded because toxicity data were lacking for metabolites and admixtures (e.g., BAM, ETU, and MITC), and for 5 out of the 20 pesticides. Only for aldicarb (including its decomposition products aldicarb-sulfon and -sulfoxide) did the  $MEC_{(max)}$  exceed the  $0.1 \cdot L(E)C_{50}$  value. None of the chemicals exceed a risk value with  $\alpha=1$ . For  $\alpha=0.01$ , risk values are exceeded for 1,3-dichloropropene and ethoprosfos. It is evident that many of the residues of pesticides detected in ground water are metabolites or admixtures, with a concentration often much higher than the original active ingredient, for which ecotoxicity data are rare or completely lacking.

Based on this preliminary ecotoxicological evaluation, Table 3 gives the pesticides and some metabolites with high potential risks for groundwater organisms (and ecosystems), which are chosen for further study with the groundwater copepod *Parastenocaris germanica*.

**Table 3.** Pesticides and metabolites selected for toxicity testing with *Parastenocaris germanica*.

|                     |                                                            |
|---------------------|------------------------------------------------------------|
| <b>Pesticides:</b>  | Propoxur, Thiram, Cypermethrin, Ethoprosfos, Fenpropathrin |
| <b>Metabolites:</b> | Aldicarb-sulfoxide, Aldicarb-sulfon, MITC, ETU             |

### 3 MATERIAL AND METHODS

#### 3.1. METHODS

Acute toxicity tests were performed according to SOP ECO/219/00. For background information see Notenboom *et al.* (1993).

##### Test animals

*Parastenocaris germanica* Kiefer, 1936 (Crustacea: Copepoda: Harpacticoida), a groundwater inhabiting microcrustacean of 400-500  $\mu\text{m}$  length, was used as a test species. The animals were collected from a bore hole near Cuyck (province of Noord-Brabant) that had access to phreatic ground water in the alluvial plain of the river Meuse. Animals were maintained in the laboratory under dark conditions at  $13 \pm 2$  °C, and in biotope water with some natural sediment and detritus, for a maximum period of 4-6 weeks. A few days before experiments, adults were transferred from sediment and detritus to petri-dishes filled with test medium for preconditioning. No selection was made for sex. From these petri-dishes, 10 active creeping adults were selected and added to each test vial with a micropipet.

##### Test design

Tests are performed in Bilthoven ground water, a raw ground water taken directly after aeration at the withdrawal station. The water is used as a drinking water supply and comes from a depth of 40-50 meters. The wells are located in a semi-natural wooded area situated at a Pleistocene pushed wall. Some physical-chemical properties of this ground water are given in Table 4. The water (test medium) was filtered (0.45  $\mu\text{m}$ ) and stored (max. 2 months) at 4 °C.

Assessment of the acute toxicity of chemicals to *P. germanica* was done in 8-16 ml vials of 2 cm in diameter closed with crimp caps which were provided with a rubber septum having the inside covered with a thin layer of teflon. Vials were placed in the dark at a temperature of  $13 \pm 2$  °C, no food was added. Because the animals creep over the bottom, their condition could easily be examined with a reverse microscope (magnification 10-20). Each vial contained preferably 10 animals, and for each concentration two or more replicates were used. Since the vials are hermetically closed, the system is safe and easy to handle. Animals were exposed to different

**Table 4.** Some physical-chemical properties of the groundwater medium used in the toxicity tests. Mean and standard deviation in the period January 1987 to July 1991 (source: WMN, Utrecht).

|                              | Mean | SD   |
|------------------------------|------|------|
| Electric conductivity (mS/m) | 21.0 | 1.13 |
| Chloride (mg/l)              | 21.3 | 2.5  |
| Sulphate (mg/l)              | 18.5 | 2.12 |
| Phosphate (mg/l)             | <1   |      |
| Hydrogen carbonate (mg/l)    | 59.2 | 7.7  |
| Nitrate (mg/l)               | 6.2  | 4.3  |
| Calcium (mg/l)               | 24.7 | 2.0  |
| Magnesium (mg/l)             | 3.5  | 0.2  |
| Iron (mg/l)                  | 0.07 | 0.05 |
| Manganese (mg/l)             | 0.13 | 0.02 |
| Total hardness (mmol/l)      | 0.75 | 0.05 |

concentrations of chemicals and after 24, 48, 72, and 96 hours, mortality and immobility were determined. In addition, a control (and control+solvent) was included.

Normally *Parastenocaris germanica* makes frequent walking movements over the bottom of the vials. Criterion for mortality was immobility, often with a dorsal concave appearance. Under the influence of certain chemicals, the animals became immobile but were still motile, i.e., occasionally their antennae or pereopods moved. This criterion of immobilization was used to assess EC<sub>50</sub>-values. Examination of each animal for at least 10 seconds was required for an appropriate judgement of the animal's condition.

#### Selection of test concentrations

Before final tests were done, preliminary tests were conducted to get an indication of the concentrations that did not cause any effect, and caused total mortality or immobility. Preliminary tests consisted of at least 5 concentrations in a geometric series with a factor of 10. Final tests were performed with at least 5 concentrations in a geometric series, preferably with a factor of 1.78 (10<sup>1/4</sup>), which should include a range of effects from 0..10 to 90..100%. The maximum toxicant concentration never exceeded 1 g/l (OECD-directive).

#### Preparation of toxicant concentrations

As a guideline for the preparation of solutions of pesticides, SOP's of the Laboratory of Organic analytical Chemistry were used (LOC/057, LOC/110 en LOC/112). When possible,

pesticides were dissolved in demi water, if necessary by ultrasonic dispersion or heating, directly followed by dilution. In case of low water solubility of the pesticides, the solvents ethanol, acetone or ethylacetate were used. Acetone and ethylacetate have a very low toxicity to *P. germanica* (Notenboom *et al.* 1993). Solvent concentrations never exceeded 0.1 g/l (OECD-standard). If stock solutions were made one day or more before the start of the test they were stored in the dark at 4 °C. The pH of the groundwater medium was measured before starting the test. After 96 hours, the end of the test, the pH in the vials was again measured by means of a micro-electrode.

#### Validity criterion

The results of the tests were considered to be valid if the percent of mortality or immobility in control groups was less than 10%; and if at any higher concentration of test substance the mortality or immobility was about equal or higher.

### 3.2. TEST SUBSTANCES AND ANALYTICAL PROCEDURES

#### Pesticides

##### **Propoxur (purity: 99%)**

Chemical family: carbamate

Biological activity: insecticide (cholinesterase inhibitor)

CAS reg. nr.: 114-26-1

Mol. weight: 209 g/mol

##### **Thiram (purity: 96%)**

Chemical family: dithiocarbamate

Biological activity: fungicide/repellent (protective leaf-fungicide and seed-dressing agent)

CAS reg. nr.: 137-26-8

Mol. weight: 240

##### **Cypermethrin (purity 96.6 %)**

Chemical family: pyrethroid

Biological activity: insecticide

CAS reg. nr.: 52315-07-8

Mol. weight: 416

**Ethoprofos (purity 99%)**

Chemical family: organophosphorous

Biological activity: nematicide/insecticide (cholinesterase inhibitor)

CAS reg. nr.: 13194-48-4

Mol. weight: 242

**Fenpropathrin (purity: 99.6%)**

Chemical family: pyrethroid

Biological activity: acaricide/insecticide

CAS reg. nr.: 64257-84-7

Mol. weight: 349

**Metabolites**

**Aldicarb-sulfoxide (purity: 99.7%)**

Metabolite of aldicarb

Chemical family: N-methylcarbamate

Biological activity: insecticide/acaricide/nematicide

CAS reg. nr.: 1646-87-3

Mol. weight: 206

**Aldicarb-sulfon (purity: 99.9%)**

Metabolite of aldicarb-sulfoxide

CAS reg. nr.: 1646-88-4

Mol. weight: 222 g/mol

**Ethylenethiourea (ETU) (purity: 98%)**

Main degradation product of ethylenebis-(dithiocarbamates), like Maneb, Zineb, Mancozeb

Chemical family: dithiocarbamates

Biological activity: fungicide

CAS reg. nr.: 96-45-7

Mol. weight: 102

**Methylisothiocyanate (MITC) (purity: 98.3%)**

Metabolite of metam-natrium.

Chemical family: isothiocyanate

Biological activity: nematocide/fungicide/herbicide

CAS reg. nr.: 556-61-6

Mol. weight: 73

### Analytical procedures

Water samples for analysis of pesticide concentrations were taken from the test vials at the end of the exposure time of 96 hours. In a few cases stock solutions were analyzed together with the other samples. Samples were stored in the dark at 4 °C. In case of Cypermethrin, Ethoprofos and Fenpropathrin, the amount of sample required for analysis (500 ml) exceeded the test vial content (8 ml). Therefore, separate stock solutions for all concentrations were made in sufficient volume, maintained under test conditions, and submitted for analysis after termination of exposure time. Propoxur samples were diluted to concentrations of 0.1 mg/l nominal in a volume of 1.6 ml with 20% acetonitril. Thiram could not be analyzed because appropriate methods were not operational when the experiments were done.

Samples of MITC and ETU were analyzed by Reversed Phase HPLC column switching. For Propoxur, Aldicarb-sulfon, and Aldicarb-sulfoxide direct HPLC techniques were used; and GC methods were applied for Cypermethrin, Ethoprofos, and Fenpropathrin. Table 5 gives an overview of the procedures of the Laboratory of Organic Analytical Chemistry (LOC) used in this project.

**Table 5.** Test compounds and reference to the chemical analytical procedures applied

|                    |                |
|--------------------|----------------|
| Propoxur           | SOP LOC/076/00 |
| Aldicarb-sulfoxide | SOP LOC/076/00 |
| Aldicarb-sulfon    | SOP LOC/076/00 |
| MITC               | SOP LOC/251/00 |
| ETU                | SOP LOC/125/00 |
| Cypermethrin       | SOP LOC/122/01 |
| Ethoprofos         | SOP LOC/123/00 |
| Fenpropathrin      | SOP LOC/122/01 |

### 3.3 STATISTICS

A log-logistic model is used for describing dose-response relationships and calculating  $LC_{50}$  and  $EC_{50}$  values. The model (LOGITNAT) is similar to that described in Notenboom *et al.* (1992), developed in GENSTAT by Dr. J.A. Hoekstra (RIVM-CWM). It describes the probability of mortality or immobility as a function of background response and exposure concentrations according a sigmoidal dose-response curve. The expected proportion of response is described as:

$$p = d + (1-d) \frac{e^{b(\ln C - a)}}{1 + e^{b(\ln C - a)}}$$

The three parameters of the model,  $a$ ,  $b$ , and  $d$  are to be estimated from the data. Parameter  $d$  is the background response rate. Parameter  $a$  is  $\ln(L(E)C_{50})$ , and  $b$  the slope of the dose-response curve in the  $L(E)C_{50}$ -point. When the model has a good fit on the data the residual mean deviance (r.m.d.) is about 1.

## 4. RESULTS

### 4.1 VALIDITY AND TEST CONDITIONS

The percentage of mortality or immobility in control groups is given in Table 6. Two tests clearly did not fulfil this validity criterion: Propoxur and MITC (1). These are rejected for further statistical analysis. In the other tests hardly any effect was recorded in control groups.

**Table 6.** Percentage of immobility in control groups after 96 hours.

| Pesticide          | Control | Control + solvent |
|--------------------|---------|-------------------|
| Propoxur           | 0       | 100               |
| Aldicarb-sulfoxide | 0       | -                 |
| Aldicarb-sulfon    | 0       | -                 |
| Thiram (1)         | 10.5    | -                 |
| Thiram (2)         | 0       | -                 |
| MITC (1)           | 50      | -                 |
| MITC (2)           | 0       | -                 |
| MITC (3)           | 5       | -                 |
| ETU                | 0       | -                 |
| Cypermethrin (1)   | 0       | 0                 |
| Cypermethrin (2)   | 0       | 0                 |
| Ethoprofos         | 6       | 0                 |
| Fenpropathrin      | 0       | 0                 |

The tests with Aldicarb-sulfoxide, Aldicarb-sulfon, Thiram (1), and MITC (3) did not fulfill the second validity criterion that at any higher concentration of test substance the mortality or immobility should be about equal or higher (see Appendix 4, 5, 6, and 10). Probably this was caused by sources of variation related to the test design (small animal numbers, small volume, unequivocal judgement of animal condition). These variation sources are difficult to reduce and partly inherent to the fact that small groundwater organisms are used (Notenboom *et al.*, 1993). Hence, it was decided not to apply this second validity criterion too severely.

Table 7 gives the pH of the groundwater medium at the start of the experiments and the mean of various measurements in vials of different concentrations at the end of the experiments. The pH at the end could not be measured in the earlier experiments because an appropriate micro-electrode was not available. In general the pH increased slightly with 0.3-0.5 units during the 96 hours of exposure time.

**Table 7.** pH at start and end of experiments. Start pH measured in medium, end pH based on mean of several (n) test vials.

| Pesticide          | pH start (medium) | pH end (mean n test vials) | n  |
|--------------------|-------------------|----------------------------|----|
| Propoxur           | 6.7               | -                          | -  |
| Aldicarb-sulfoxide | 7.4               | -                          | -  |
| Aldicarb-sulfon    | 7.4               | -                          | -  |
| Thiram (1)         | 7                 | -                          | -  |
| Thiram (2)         | 7.3               | 7.8                        | 6  |
| MITC (1)           | 7                 | -                          | -  |
| MITC (2)           | 7                 | -                          | -  |
| MITC (3)           | 7.4               | 7.7                        | 7  |
| ETU                | 7.2               | 7.7                        | 5  |
| Cypermethrin (1)   | 7.4               | 7.8                        | 4  |
| Cypermethrin (2)   | 7.2               | 7.6                        | 7  |
| Ethoprosfos        | 7.2               | 7.6                        | 11 |
| Fenpropathrin      | 7.3               | 7.6                        | 7  |

#### 4.2 ACTUAL VERSUS NOMINAL CONCENTRATIONS

It was not always possible to start sample treatment and analysis in the week directly following the end of experiments. Therefore, storage time of water samples prior to analysis differed between experiments. In Table 8, the end dates of the experiments and starting dates of chemical analysis are given. In several experiments, storage time of samples was longer than three weeks.

**Table 8.** History of samples for analysis after termination of experiments.

| Experiment         | End date | Date analysis | Storage time (days) |
|--------------------|----------|---------------|---------------------|
| Propoxur           | 15-03-91 | -             | -                   |
| Aldicarb-sulfoxide | 26-06-92 | 29-06-92      | 3                   |
| Aldicarb-sulfon    | 26-06-92 | 29-06-92      | 3                   |
| MITC (1)           | 25-10-91 | 29-10-92      | 4                   |
| MITC (2)           | 20-03-92 | 08-04-92      | 19                  |
| MITC (3)           | 18-12-92 | 23-12-92      | 5                   |
| ETU                | 13-04-92 | 16-06-92      | 64                  |
| Cypermethrin (1)   | 17-04-92 | ca. 01-06-92  | ca. 45              |
| Cypermethrin (2)   | 30-04-93 | 27-05-93      | 27                  |
| Ethoprosfos        | 19-04-93 | ca. 15-08-93  | ca. 118             |
| Fenpropathrin      | 10-05-93 | 12-08-93      | 94                  |

**Table 9.** Results of stock solution analysis

| Compound (exp) mg/l | Nominal | Actual | % deviation | days after preparation |
|---------------------|---------|--------|-------------|------------------------|
| MITC (2)            | 24.4    | 25     | +2.5        | 26                     |
| Ethoprofos          | 17.36   | 14.52  | -16         | ca. 118                |
| Fenpropathrin       | 96      | 78.7   | -18         | 94                     |

Results of actual concentration measurements if available are given in appendices 3 to 15. The results of stock solution analysis are given in Table 9. In case of both Cypermethrin experiments, the peak pattern found with HPLC did not correspond to that of the analytical standards. Nevertheless, the performance of animals in the experiments showed a clear dose-response relationship. Measured and nominal concentrations of the MITC (2) experiment showed little similarity. This was because of volatilization of the compound from samples which were unfortunately stored in poorly closed vials. A difference larger than 20% (OECD-criterion) between nominal and measured concentrations was seen in Propoxur, Aldicarb-sulfon, MITC (3), Ethoprofos, and Fenpropathrin experiments. Nominal and measured concentrations differed less than 20% in Aldicarb-sulfoxide, MITC (1), and ETU experiments. In order to be able to compare experiments with unknown differences in dissipation of pesticides from the test system or during sample storage,  $L(E)C_{50}$ -values are expressed as initial concentrations, based on nominal values. Since experiments were performed by an experienced technician, the chance of errors made during preparation of stock solutions and test concentrations is considered small.

### 4.3 LETHALITY

$LC_5$ - and  $LC_{50}$ -values calculated from valid test results are given in Table 10. Results of the test with ETU did not permit calculations because in the highest concentration (1 mg/l) almost no mortality of organisms occurred. This substance is only slightly toxic for *P. germanica* and it appeared unrealistic to repeat the experiment with a range of higher concentrations. The r.m.d. of the log-logistic model based on Thiram (2) data is rather high because of large residuals, this in contrary to the other models which show r.m.d. values  $\leq 1.6$ . The slope in the  $LC_{50}$ -point of the

dose-response curve of Ethoprofos is very steep, and as a consequence there is a relatively small difference between the  $LC_5$  and  $LC_{50}$ .  $LC_{50}$ -values of repeated experiments with Cypermethrin showed a difference of about a factor 4, and in both cases the r.m.d. was about 1.

**Table 10.**  $LC_5$ - and  $LC_{50}$ -values (95% confidence interval), model parameters (a and b), and residual mean deviance (r.m.d.) from the log-logistic model for valid test results after 96 hours of exposure.

| Pesticide ( $\mu\text{g/l}$ ) | $LC_5$            | $LC_{50}$         | a     | b    | r.m.d |
|-------------------------------|-------------------|-------------------|-------|------|-------|
| Thiram (2)                    | 0.23 (0.08-0.68)  | 4.40 (1.75-11.09) | 1.48  | 1    | 7.46  |
| MITC (2)                      | 69 (39-125)       | 176 (140- 221)    | 5.17  | 3.16 | 0.73  |
| ETU                           | -                 | -                 | -     | -    | -     |
| Cypermethrin (1)              | 0.01 (0.001-0.11) | 0.40 (0.24-0.68)  | -0.92 | 0.8  | 1.07  |
| Cypermethrin (2)              | 0.08 (0.03-0.22)  | 1.78 (1.09-2.91)  | 0.58  | 0.95 | 0.98  |
| Ethoprofos                    | 497 (471-524)     | 568 (543-595)     | 6.34  | 22   | 1.42  |
| Fenpropathrin                 | 0.20 (0.07-0.52)  | 1.97 (1.25-3.11)  | 0.98  | 1.3  | 1.59  |

#### 4.4 IMMOBILITY

The categories of immobile and death (Appendices 3-15) were taken together for calculating  $EC_5$ - and  $EC_{50}$ -values (Table 11). In case of Cypermethrin (1) no EC-values could be estimated due to the absence of any partial response (Appendix 12). The r.m.d. values strongly deviate from one. The value for Thiram (2) is rather high, those for MITC (2), ETU, Cypermethrin (2), and Fenpropathrin extremely low. Only the Ethoprofos value is around the desired value of one.

**Table 11.**  $EC_5$ - and  $EC_{50}$ -values (95% confidence interval) for immobility, model parameters (a and b), and residual mean deviance (r.m.d.) from the log-logistic model for valid test results after 96 hours of exposure.

| Pesticide ( $\mu\text{g/l}$ ) | $EC_5$              | $EC_{50}$           | a     | b     | r.m.d.   |
|-------------------------------|---------------------|---------------------|-------|-------|----------|
| Thiram (2)                    | 0.23 (0.08-0.62)    | 2.25 (1.28-3.95)    | 0.81  | 1.28  | 12.61    |
| MITC (2)                      | 30.8 (30.4-31.1)    | 31.9 (31.5-32.2)    | 3.46  | 84.19 | 0.000041 |
| ETU                           | 57 (8-413)          | 2330 (440-12270)    | 0.85  | 0.79  | 0.09     |
| Cypermethrin (2)              | 0.01 (0.006-0.016)  | 0.019 (0.015-0.025) | -3.95 | 4.62  | 0.053    |
| Ethoprofos                    | 326 (219-486)       | 450 (371-544)       | 6.11  | 9.18  | 1.05     |
| Fenpropathrin                 | 0.003 (0.002-0.005) | 0.006 (0.005-0.008) | -5.08 | 4.58  | 0.003    |

Attention is called to the large confidence intervals of the EC values of ETU.

#### 4.5 INTEGRATION AND EVALUATION OF RESULTS

An overview of best estimates of  $LC_{50}$  and  $EC_{50}$  values are given in Table 12. Thiram and the two pyrethroids, Cypermethrin and Fenpropathrin, appeared to be the most toxic to *P. germanica*. No good estimates could be made of toxicity of all degradation products. Nevertheless, MITC appeared the most toxic of these products. The difference between  $LC_{50}$  and  $EC_{50}$  value appeared especially large in the cases of Cypermethrin and Fenpropathrin ( $EC_{50}/LC_{50}$ -ratio small). Both compounds are pyrethroids with a non-systemic mode of action.

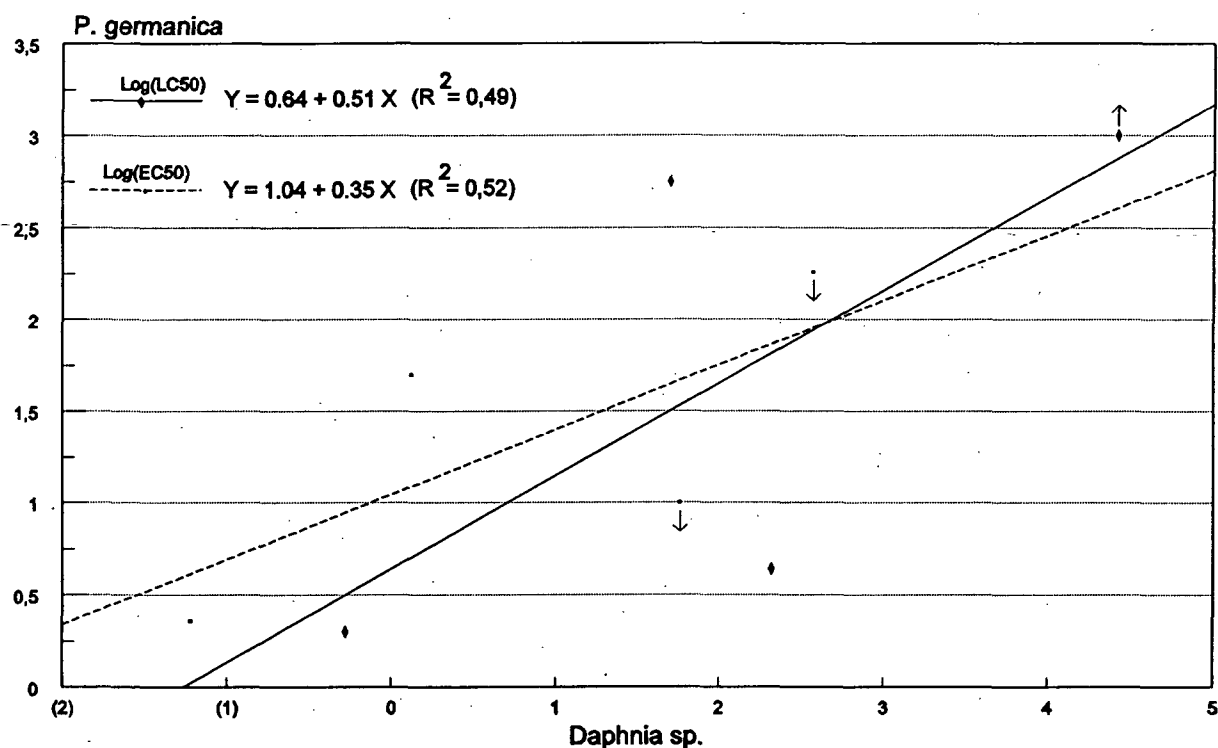
Comparison of *P. germanica* toxicity data with available information from tests with *Daphnia* species (Table 13) is limited by the incompleteness of both data sets. For the case of the *P. germanica* the  $LC_{50}$  value of ETU is replaced by the lowest point, and for the  $EC_{50}$  values of the Aldicarb metabolites by the highest points of the indicated range. An intraspecific comparison based on four-values is possible. As Figure 1 shows there appeared to be a weak relation between the toxicity of the pesticides (incl. metabolites) to both species. However, a consistent difference in sensitivity between the two species does not seem to exist.

**Table 12.** Review of conservative estimates of L(E) $C_{50}$ -values (96 hours) for *Parastenocaris germanica* ( $\mu\text{g/l}$ ).

| Pesticide          | $LC_{50}$ | $EC_{50}$ | $EC_{50}/LC_{50}$ |
|--------------------|-----------|-----------|-------------------|
| Aldicarb-sulfoxide | -         | <10       | -                 |
| Aldicarb-sulfon    | -         | <180      | -                 |
| Thiram             | 4.4       | 2.3       | 0.52              |
| MITC               | 176       | 32        | 0.18              |
| ETU                | >1000     | 2330      | -                 |
| Cypermethrin       | 1.1       | 0.02      | 0.002             |
| Ethoprofos         | 568       | 450       | 0.79              |
| Fenpropathrin      | 2         | 0.006     | 0.003             |

**Table 13.** Review of L(E)C<sub>50</sub>-values (48-96 hours) for *Daphnia* spec. (µg/l).

| Pesticide (µg/l)   | LC <sub>50</sub> | EC <sub>50</sub> | Species                  | Reference                        |
|--------------------|------------------|------------------|--------------------------|----------------------------------|
| Aldicarb-sulfoxide | 103              | 57               | <i>D. laevis</i> (adult) | Foran <i>et al.</i> , 1985       |
| Aldicarb-sulfon    | 1124             | 369              | <i>D. laevis</i> (adult) | Foran <i>et al.</i> , 1985       |
| Thiram             | 210              | 0.06             | <i>D. magna</i>          | Van Leeuwen <i>et al.</i> 1985   |
| MITC               | -                | -                | -                        | -                                |
| ETU                | 26000            | -                | <i>D. magna</i>          | Van Leeuwen <i>et al.</i> , 1985 |
| Cypermethrin       | -                | 1.3              | <i>D. magna</i>          | RIVM, 1991 <sup>a</sup>          |
| Ethoprofos         | 50               | -                | <i>D. magna</i>          | RIVM, 1989                       |
| Fenpropathrin      | 0.53             | -                | <i>D. magna</i> (juvs.)  | RIVM, 1991 <sup>b</sup>          |

**Figure 1.** Logarithmic transformed LC<sub>50</sub> and EC<sub>50</sub> data (n=4) of *Daphnia* sp. plotted against *P. germanica* and linear regression lines (equations). Arrows indicate toxicity ranges and their directions.

## 5. DISCUSSION AND CONCLUSION

The toxicity of nine different pesticides or degradation products with a potential risk for groundwater metazoans was investigated. In total 13 different toxicity experiments were performed using as the test species the groundwater copepod *P. germanica*. Mortality and immobility were chosen as toxicological end points. With the exception of two experiments, there were no signs of reduced performance of test animals in the control groups. This small percentage of mortality/immobility observed in control groups is in agreement with earlier experience with this experimental design (Notenboom *et al.*, 1993). The pH of the test medium remained constant within 0.3-0.5 units during the 96 hours of exposure time. Nevertheless, other sources of variance appear to be present because some results showed indistinct dose-response relationships, with the percentage of mobility/immobility not equal or higher at increasing doses [Thiram and MITC (3)], or poorly reproducible results [Cypermethrin (1) and (2)].

Also, earlier experience showed that considerable sources of variance may exist, probably partly related to the technical design of the test and partly to heterogeneity of the biological starting material. Some recommendations to reduce uncertainties were previously formulated (Notenboom *et al.*, 1993). Biological variation in sensitivity between experiments, may have been caused by test animals coming from a field population. The field population is sampled at different times under distinct field conditions. Field populations, in general, are genetically more heterogeneous and more dynamic than standardized laboratory cultures. Theoretically this may cause sensitivity differences between batches of test animals originating from different dates.

Technical variation sources may be related to the small volume of test media, small number and small size of test organisms, and errors made during execution of tests (e.g., by the preparation of stock and test solutions). Counting and equivocal judgment of the animal's condition may also be a source of error.

No good measurements of toxic compound concentrations in the test vials after exposure time were obtained in all experiments. For Thiram and MITC (2), no measurements are available at all. In case of Cypermethrin, the peak pattern found by HPLC did not correspond with that of analytical standards. Degradation of Cypermethrin during storage is a possible explanation. Dissipation of test substances (e.g., through hydrolysis or biodegradation) during the tests or afterwards during storage of samples cannot be excluded and might account for differences of

more than 20% found between nominal and measured concentrations (Aldicarb-sulfon, MITC (3), Ethoprofos, and Fenpropathrin). Because experiments were performed by an experienced technician, and the stock solution concentrations measured were similar to nominal values, the nominal values of test substances were considered to be good estimates of initial concentrations. Test results are therefore expressed in initial concentrations. This approach seems to be justified in order to reduce the contribution of dissipation in observed differences in pesticide toxicity to *P. germanica*.

The most toxic to *P. germanica* are Fenpropathrin and Cypermethrin, followed by Thiram; moderately toxic are Aldicarb-sulfon, Aldicarb-sulfoxide, MITC, and Ethoprofos; and the least toxic is ETU. The large difference between the  $LC_{50}$  and  $EC_{50}$  values of Cypermethrin and Fenpropathrin is remarkable. It is known that Cypermethrin also acts on the central and peripheral nervous system at very low doses (Anonymous, 1994). This may explain why immobilization takes place at a very low dose, as it does for the chemically similar Fenpropathrin. Comparison of pesticide toxicity data of *P. germanica* with available information from *Daphnia* sp. did not reveal a very consistent picture. For some compounds *P. germanica* appears to be more sensitive than *Daphnia* sp., for others it is the reverse. Differences observed between *P. germanica* and *Daphnia* sp. are within a range of about 4 to 60, hence less than two orders of magnitude.

Pesticides used in this study were selected based on a potential hazard screening for groundwater metazoans. Results of the study now enable estimation of preliminary risks based on *P. germanica* toxicity data according to the modified EPA method (Slooff, 1992). Following this method an assessment factor of 1000 is recommended in order to derive ecotoxicologically safe environmental concentrations in case acute toxicity data for only one species is available (Table 11). It is interesting to note that the indicative maximum tolerable concentrations for most of the compounds (exceptions are Aldicarb-sulfon, ETU, and Ethoprofos) are lower than the actual standard of 0.1  $\mu\text{g/l}$  for pesticides in ground water. When indicative maximum tolerable concentration are compared with environmental concentrations, according to worst case scenario's, than this value is exceeded for all compounds except ETU (Table 14). These results are not surprising because these pesticides were suspected of being potentially harmful for crustaceans, and no consistent difference in ecotoxicological profile, based on acute toxicities, seems to exist between the groundwater *P. germanica* and the surface water *Daphnia*, which are both crustaceans. The use of aquatic toxicity data in preliminary ecotoxicological effect assess-

**Table 14.** Preliminary effect assessment procedure for groundwater according to Slooff (1992) based on *P. germanica* toxicity data. Environmental Concentration (EC) are or predicted  $PEC_{(max)}$  values by PESTLA (worst case scenario) or based on available measurements  $MEC_{(max)}$  (highest values taken) (see section 2). In bold indicative maximum tolerable concentrations below the actual groundwater standard for pesticides of  $0.1 \mu\text{g/l}$ .

| Pesticide ( $\mu\text{g/l}$ ) | L(E) $C_{50}$ | indicative maximum tolerable concentration |                     |
|-------------------------------|---------------|--------------------------------------------|---------------------|
|                               |               | L(E) $C_{50}$ *0.001                       | EC <sub>(max)</sub> |
| Aldicarb-sulfoxide            | <10           | <b>&lt;0.01</b>                            | 130*                |
| Aldicarb-sulfon               | <180          | <b>&lt;0.18</b>                            | 130*                |
| Thiram                        | 2.3           | <b>0.0023</b>                              | 50                  |
| MITC                          | 32            | <b>0.032</b>                               | 0.6                 |
| ETU                           | 2330          | 2.3                                        | 0.4                 |
| Cypermethrin                  | 0.02          | <b>0.00002</b>                             | 0.088               |
| Ethoprofos                    | 450           | 0.45                                       | 40                  |
| Fenpropathrin                 | 0.006         | <b>0.000006</b>                            | 23.8                |

\* Sum concentration of Aldicarb, incl. metabolites.

ment for groundwater ecosystems as proposed by Slooff (1992) seems to be justified according to the results obtained in this study.

In discussions on acceptable levels of pesticides in ground water, little or no attention is given to the side effects that agrochemicals may have on groundwater organisms and ecosystems. A crucial question is whether or not existing standards of pesticides in groundwater sufficiently protect the ecological integrity of that ecosystem. The results of this study indicate that this is not the case for all pesticides.

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## Appendix 1:

Some physical-chemical properties of the biotope water (St. Agatha, Cuyck) of the *P. germanica* population used for this study. Analysis performed by the RIVM Laboratory of Anorganic Analytical Chemistry (LAC).

| parameter          | unity | sampling date    |                |        |         |         |
|--------------------|-------|------------------|----------------|--------|---------|---------|
|                    |       | 14/6/89          | 20/9/89        | 2/8/90 | 16/5/91 | 15/5/92 |
| pH                 |       | 6.4              | 4.9            | 5.8    | 4.9     | 4.9     |
| Temperature        | °C    | 10.4             | - <sup>1</sup> | -      | -       | -       |
| Conductivity       | µS/cm | 206              | 528            | 431    | 520     | 505     |
| Oxygen             | mg/l  | 2.6 <sup>2</sup> | -              | -      | -       | -       |
| Chloride           | mg/l  | 14.7             | 46.0           | 33.3   | 42.97   | 40.9    |
| Sulphate           | mg/l  | 34.9             | 112.9          | 88.6   | 107.6   | 98.8    |
| Phosphate          | µg/l  | 526              | 19             | 75     | < 10    | -       |
| Total phosphor     | mg/l  | 0.36             | <0.06          | <0.06  | <0.06   | < 0.06  |
| Hydrogen carbonate | mg/l  | 40               | 3              | 10     | < 3     | < 3     |
| Nitrate            | mg/l  | 16               | 90             | 84     | 96.8    | 96.8    |
| Ammonium           | mg/l  | 0.58             | 0.05           | 0.1    | < 0.02  | 0.02    |
| Potassium          | mg/l  | 4.7              | 4.8            | 4.8    | 4.8     | 4.7     |
| Calcium            | mg/l  | 62.3             | 63.1           | 53.6   | 60.9    | 61.3    |
| Magnesium          | mg/l  | 10.7             | 11.4           | 7.7    | 9.4     | 9.0     |
| Sodium             | mg/l  | 25.1             | 24.5           | 20.1   | 25.4    | 24.2    |
| Iron               | µg/l  | 64               | 579            | 41     | 78      | 329.5   |
| zinc               | µg/l  | -                | -              | -      | -       | 104.6   |
| Aluminium          | µg/l  | -                | -              | -      | -       | 526.5   |
| Manganese          | µg/l  | -                | -              | -      | -       | 607.6   |
| DOC                | mg/l  | 1.2              | 0.9            | 2.0    | 1.2     | 0.98    |

1: no analysis performed;

2: in situ measurement after water extraction.

Appendix 2: Some physical-chemical properties of the groundwater medium used in the toxicity tests. Mean and standard deviation in the period January 1987 to July 1991 (Source: N.V. Waterleidingbedrijf Midden-Nederland).

| Parameter                       | Eenheid              | Gem.  | SD   |
|---------------------------------|----------------------|-------|------|
| pH                              | pH                   | 6.9   | 0.12 |
| Temperature                     | °C                   | 10.7  | 0.45 |
| Conductivity                    | mS/m                 | 21.0  | 1.13 |
| Oxygen <sup>1</sup>             | mg/l                 | 1.38  | 0.33 |
| Free carbondioxide <sup>2</sup> | mg/l                 | 14.1  | 2.7  |
| Chloride                        | mg/l                 | 21.3  | 2.5  |
| Sulphate                        | mg/l                 | 18.5  | 2.12 |
| Phosphate                       | mg/l                 | <1    |      |
| Hydrogen carbonate              | mg/l                 | 59.2  | 7.7  |
| Carbonate                       | mg/l                 | <1    |      |
| Total hardness <sup>3</sup>     | mmol/l               | 0.75  | 0.05 |
| Nitrate                         | mg/l                 | 6.22  | 4.3  |
| Nitrite                         | mg/l                 | <0.01 |      |
| Ammonium                        | mg/l                 | 0.16  | 0.04 |
| Calcium                         | mg/l                 | 24.7  | 2.00 |
| Magnesium                       | mg/l                 | 3.46  | 0.2  |
| Iron                            | mg/l                 | 0.07  | 0.05 |
| Manganese                       | mg/l                 | 0.13  | 0.02 |
| Kaliumpermanganate oxidation    | mg O <sub>2</sub> /l | 0.53  | 0.39 |

1: Jodometric determination afters Winkler (NEN-standard 6632).

2: Titrimetric determination (NEN-standard 6486) (Analysis of oxygen free carbondioxide directly after sampling).

3: Total concentrations of Calcium and Magnesium.

Appendix 3: Influence of Propoxur on the mortality (%) en mobility (%) of *P. germanica* after 24, 48, 72 and 96 hours of exposure.

Animal capture date: 28-2-91.

Date: 11-3-91. Responsible technician: J.J. Boessenkool.

| Propoxur (mg/l)  |                   | time | pH | n <sup>1</sup> | mobile |    | immobile |   | death |    |
|------------------|-------------------|------|----|----------------|--------|----|----------|---|-------|----|
| nom <sup>2</sup> | actu <sup>3</sup> | (hr) |    |                | 1      | 2  | 1        | 2 | 1     | 2  |
| 0                | 0.0               | 24   |    | 20             | 10     | 10 | 0        | 0 | 0     | 0  |
|                  |                   | 48   |    | 20             | 10     | 10 | 0        | 0 | 0     | 0  |
|                  |                   | 72   |    | 20             | 10     | 10 | 0        | 0 | 0     | 0  |
|                  |                   | 96   |    | 20             | 10     | 10 | 0        | 0 | 0     | 0  |
| 0 <sup>4</sup>   | 0.0               | 24   |    | 20             | 10     | 10 | 0        | 0 | 0     | 0  |
|                  |                   | 48   |    | 20             | 10     | 10 | 0        | 0 | 0     | 0  |
|                  |                   | 72   |    | 20             | 0      | 0  | 0        | 0 | 10    | 10 |
|                  |                   | 96   |    | 20             | 0      | 0  | 0        | 0 | 10    | 10 |
| 0.01             | 0.013             | 24   |    | 20             | 10     | 10 | 0        | 0 | 0     | 0  |
|                  |                   | 48   |    | 20             | 10     | 10 | 0        | 0 | 0     | 0  |
|                  |                   | 72   |    | 20             | 10     | 10 | 0        | 0 | 0     | 0  |
|                  |                   | 96   |    | 20             | 10     | 10 | 0        | 0 | 0     | 0  |
| 0.1              | 0.15              | 24   |    | 21             | 11     | 10 | 0        | 0 | 0     | 0  |
|                  |                   | 48   |    | 21             | 11     | 10 | 0        | 0 | 0     | 0  |
|                  |                   | 72   |    | 21             | 0      | 0  | 0        | 0 | 11    | 10 |
|                  |                   | 96   |    | 21             | 0      | 0  | 0        | 0 | 11    | 10 |
| 1.0              | 1.3               | 24   |    | 20             | 10     | 9  | 0        | 0 | 0     | 1  |
|                  |                   | 48   |    | 20             | 10     | 9  | 0        | 0 | 0     | 1  |
|                  |                   | 72   |    | 20             | 0      | 0  | 0        | 0 | 10    | 10 |
|                  |                   | 96   |    | 20             | 0      | 0  | 0        | 0 | 10    | 10 |
| 10.0             | 16.5              | 24   |    | 19             | 10     | 9  | 0        | 0 | 0     | 0  |
|                  |                   | 48   |    | 19             | 10     | 9  | 0        | 0 | 0     | 0  |
|                  |                   | 72   |    | 19             | 0      | 0  | 0        | 0 | 10    | 9  |
|                  |                   | 96   |    | 19             | 0      | 0  | 0        | 0 | 10    | 9  |

1: total number of specimens in the duplicates;

2: nominal concentration (mg/l);

3: actual concentration (mg/l).

4: solvent ethanol, 0.001% in 0+.

Appendix 4: Influence of Aldicarb-sulfoxide on the mortality (%) en mobility (%) of *P. germanica* after 24, 48, 72 and 96 hours of exposure.

Animal capture date: 04-06-92.

Date: 22-06-92. Responsible technician: J.J. Boessenkool.

| Aldicarb-sulfoxide (mg/l) |                   | time | pH | n <sup>1</sup> | mobile |    | immobile |    | death |   |
|---------------------------|-------------------|------|----|----------------|--------|----|----------|----|-------|---|
| nom <sup>2</sup>          | actu <sup>3</sup> | (hr) |    |                | 1      | 2  | 1        | 2  | 1     | 2 |
| 0                         | 0.0               | 24   |    | 20             | 10     | 10 | 0        | 0  | 0     | 0 |
|                           |                   | 48   |    | 20             | 10     | 10 | 0        | 0  | 0     | 0 |
|                           |                   | 72   |    | 20             | 10     | 10 | 0        | 0  | 0     | 0 |
|                           |                   | 96   |    | 20             | 10     | 10 | 0        | 0  | 0     | 0 |
| 0.01                      | 0.012             | 24   |    | 19             | 0      | 0  | 9        | 9  | 0     | 1 |
|                           |                   | 48   |    | 19             | 0      | 0  | 8        | 6  | 1     | 4 |
|                           |                   | 72   |    | 19             | 0      | 0  | 6        | 5  | 3     | 5 |
|                           |                   | 96   |    | 19             | 0      | 0  | 6        | 5  | 3     | 5 |
| 0.018                     | 0.018             | 24   |    | 20             | 0      | 0  | 7        | 4  | 3     | 6 |
|                           |                   | 48   |    | 20             | 0      | 0  | 4        | 3  | 6     | 7 |
|                           |                   | 72   |    | 20             | 0      | 0  | 4        | 3  | 6     | 7 |
|                           |                   | 96   |    | 20             | 0      | 0  | 3        | 3  | 7     | 7 |
| 0.032                     | 0.031             | 24   |    | 22             | 0      | 0  | 11       | 9  | 1     | 1 |
|                           |                   | 48   |    | 22             | 0      | 0  | 9        | 7  | 3     | 3 |
|                           |                   | 72   |    | 22             | 0      | 0  | 9        | 3  | 3     | 7 |
|                           |                   | 96   |    | 22             | 0      | 0  | 7        | 3  | 5     | 7 |
| 0.056                     | 0.053             | 24   |    | 16             | 0      | 0  | 6        | 8  | 1     | 1 |
|                           |                   | 48   |    | 16             | 0      | 0  | 4        | 8  | 5     | 2 |
|                           |                   | 72   |    | 16             | 0      | 0  | 2        | 7  | 7     | 3 |
|                           |                   | 96   |    | 16             | 0      | 0  | 2        | 7  | 7     | 3 |
| 0.1                       | 0.093             | 24   |    | 20             | 0      | 0  | 9        | 9  | 1     | 1 |
|                           |                   | 48   |    | 20             | 0      | 0  | 9        | 9  | 1     | 1 |
|                           |                   | 72   |    | 20             | 0      | 0  | 9        | 8  | 1     | 2 |
|                           |                   | 96   |    | 20             | 0      | 0  | 9        | 7  | 1     | 3 |
| 0.18                      | 0.17              | 24   |    | 20             | 0      | 0  | 10       | 10 | 0     | 0 |
|                           |                   | 48   |    | 20             | 0      | 0  | 10       | 10 | 0     | 0 |
|                           |                   | 72   |    | 20             | 0      | 0  | 10       | 10 | 0     | 0 |
|                           |                   | 96   |    | 20             | 0      | 0  | 10       | 10 | 0     | 0 |

1: total number of specimens in the duplicates;

2: nominal concentration (mg/l);

3: actual concentration (mg/l).

Appendix 5: Influence of Aldicarb-sulfon on the mortality (%) en mobility (%) of *P. germanica* after 24, 48, 72 and 96 hours of exposure.

Animal capture date: 04-06-92.

Date: 22-06-92. Responsible technician: J.J. Boessenkool.

| Aldicarb-sulfon (mg/l) |                   | time | pH | n <sup>1</sup> | mobile |    | immobile |   | death |   |
|------------------------|-------------------|------|----|----------------|--------|----|----------|---|-------|---|
| nom <sup>2</sup>       | anal <sup>3</sup> | (hr) |    |                | 1      | 2  | 1        | 2 | 1     | 2 |
| 0                      | 0.0               | 24   |    | 20             | 10     | 10 | 0        | 0 | 0     | 0 |
|                        |                   | 48   |    | 20             | 10     | 10 | 0        | 0 | 0     | 0 |
|                        |                   | 72   |    | 20             | 10     | 10 | 0        | 0 | 0     | 0 |
|                        |                   | 96   |    | 20             | 10     | 10 | 0        | 0 | 0     | 0 |
| 0.01                   | 0.005             | 24   |    | 18             | 8      | 8  | 0        | 0 | 0     | 2 |
|                        |                   | 48   |    | 18             | 0      | 0  | 8        | 7 | 0     | 3 |
|                        |                   | 72   |    | 18             | 0      | 0  | 7        | 8 | 3     | 0 |
|                        |                   | 96   |    | 18             | 0      | 0  | 7        | 7 | 1     | 3 |
| 0.018                  | 0.008             | 24   |    | 21             | 11     | 10 | 0        | 0 | 0     | 0 |
|                        |                   | 48   |    | 21             | 6      | 5  | 5        | 5 | 0     | 0 |
|                        |                   | 72   |    | 21             | 7      | 6  | 4        | 3 | 0     | 1 |
|                        |                   | 96   |    | 21             | 5      | 6  | 6        | 3 | 0     | 1 |
| 0.032                  | 0.018             | 24   |    | 19             | 10     | 9  | 0        | 0 | 0     | 0 |
|                        |                   | 48   |    | 19             | 10     | 9  | 0        | 0 | 0     | 0 |
|                        |                   | 72   |    | 19             | 10     | 9  | 0        | 0 | 0     | 0 |
|                        |                   | 96   |    | 19             | 9      | 9  | 1        | 0 | 0     | 0 |
| 0.056                  | 0.029             | 24   |    | 20             | 10     | 9  | 0        | 1 | 0     | 0 |
|                        |                   | 48   |    | 20             | 8      | 8  | 2        | 2 | 0     | 0 |
|                        |                   | 72   |    | 20             | 8      | 8  | 2        | 2 | 0     | 0 |
|                        |                   | 96   |    | 20             | 8      | 8  | 2        | 2 | 0     | 0 |
| 0.1                    | 0.051             | 24   |    | 18             | 10     | 8  | 0        | 0 | 0     | 0 |
|                        |                   | 48   |    | 18             | 10     | 8  | 0        | 0 | 0     | 0 |
|                        |                   | 72   |    | 18             | 10     | 8  | 0        | 0 | 0     | 0 |
|                        |                   | 96   |    | 18             | 10     | 8  | 0        | 0 | 0     | 0 |
| 0.18                   | 0.091             | 24   |    | 20             | 0      | 0  | 5        | 8 | 5     | 2 |
|                        |                   | 48   |    | 20             | 0      | 0  | 4        | 8 | 6     | 2 |
|                        |                   | 72   |    | 20             | 0      | 0  | 3        | 7 | 7     | 3 |
|                        |                   | 96   |    | 20             | 0      | 0  | 3        | 7 | 7     | 3 |

1: total number of specimens in the duplicates;

2: nominal concentration (mg/l);

3: actual concentration (mg/l).

Appendix 6: Influence of Thiram (1) on the mortality (%) en mobility (%) of *P. germanica* after 24, 48, 72 and 96 hours of exposure.

Animal capture date: 09-09-91.

Date: 09-09-91. Responsible technician: J.J. Boessenkool.

| Thiram ( $\mu\text{g/l}$ ) |                  | time | pH  | n <sup>1</sup> | Mobile |    | Immobile |    | Death |    |
|----------------------------|------------------|------|-----|----------------|--------|----|----------|----|-------|----|
| nom <sup>2</sup>           | act <sup>3</sup> | (hr) |     |                | 1      | 2  | 1        | 2  | 1     | 2  |
| 0                          |                  | 24   | 7.0 | 19             | 9      | 10 | 0        | 0  | 0     | 0  |
|                            |                  | 48   |     | 19             | 9      | 10 | 0        | 0  | 0     | 0  |
|                            |                  | 72   |     | 19             | 7      | 10 | 2        | 0  | 0     | 0  |
|                            |                  | 96   |     | 19             | 7      | 10 | 2        | 0  | 0     | 0  |
| 1,8                        |                  | 24   | 7.0 | 20             | 3      | 6  | 7        | 4  | 0     | 0  |
|                            |                  | 48   |     | 19             | 0      | 2  | 10       | 6  | 0     | 1  |
|                            |                  | 72   |     | 20             | 1      | 0  | 7        | 10 | 2     | 0  |
|                            |                  | 96   |     | 19             | 0      | 0  | 4        | 5  | 6     | 5  |
| 3,2                        |                  | 24   | 7.0 | 20             | 11     | 6  | 0        | 3  | 0     | 0  |
|                            |                  | 48   |     | 20             | 11     | 7  | 0        | 2  | 0     | 0  |
|                            |                  | 72   |     | 20             | 11     | 8  | 0        | 1  | 0     | 0  |
|                            |                  | 96   |     | 20             | 11     | 8  | 0        | 1  | 0     | 0  |
| 5,6                        |                  | 24   |     | 17             | 1      | 0  | 5        | 7  | 2     | 2  |
|                            |                  | 48   |     | 18             | 0      | 0  | 1        | 0  | 7     | 10 |
|                            |                  | 72   |     | 18             | 0      | 0  | 0        | 0  | 8     | 10 |
|                            |                  | 96   |     | 18             | 0      | 0  | 0        | 0  | 8     | 10 |
| 10                         |                  | 24   |     | 21             | 0      | 0  | 1        | 3  | 10    | 7  |
|                            |                  | 48   |     | 21             | 0      | 0  | 0        | 0  | 11    | 10 |
|                            |                  | 72   |     | 21             | 0      | 0  | 0        | 0  | 11    | 10 |
|                            |                  | 96   |     | 21             | 0      | 0  | 0        | 0  | 11    | 10 |
| 32                         |                  | 24   |     | 2              | 0      | 0  | 4        | 4  | 6     | 6  |
|                            |                  | 48   |     | 20             | 0      | 0  | 0        | 0  | 10    | 10 |
|                            |                  | 72   |     | 20             | 0      | 0  | 0        | 0  | 10    | 10 |
|                            |                  | 96   |     | 20             | 0      | 0  | 0        | 0  | 10    | 10 |

1: total number of specimens in the duplicates;

2: nominal concentration ( $\mu\text{g/l}$ );

3: actual concentration ( $\mu\text{g/l}$ )

Appendix 7: Influence of Thiram (2) on the mortality (%) en mobility (%) of *P. germanica* after 24, 48, 72 and 96 hours of exposure.

Animal capture date: 16-01-92.

Date: 20-01-92. Responsible technician: J.J. Boessenkool.

| Thiram ( $\mu\text{g/l}$ ) |                  | Time | pH  | n <sup>1</sup> | Mobile |    | Immobile |   | Death |   |
|----------------------------|------------------|------|-----|----------------|--------|----|----------|---|-------|---|
| nom <sup>2</sup>           | act <sup>3</sup> | (hr) |     |                | 1      | 2  | 1        | 2 | 1     | 2 |
| 0                          | -                | 24   | 7.4 | 20             | 10     | 10 | 0        | 0 | 0     | 0 |
|                            |                  | 48   |     | 20             | 10     | 10 | 0        | 0 | 0     | 0 |
|                            |                  | 72   |     | 20             | 10     | 10 | 0        | 0 | 0     | 0 |
|                            |                  | 96   | 7.9 | 20             | 10     | 10 | 0        | 0 | 0     | 0 |
| 0,1                        | -                | 24   | 7.3 | 19             | 9      | 10 | 0        | 0 | 0     | 0 |
|                            |                  | 48   |     | 19             | 8      | 10 | 0        | 0 | 1     | 0 |
|                            |                  | 72   |     | 19             | 8      | 10 | 0        | 0 | 1     | 0 |
|                            |                  | 96   |     | 19             | 8      | 10 | 0        | 0 | 1     | 0 |
| 0,18                       | -                | 24   |     | 18             | 8      | 10 | 0        | 0 | 0     | 0 |
|                            |                  | 48   |     | 18             | 8      | 10 | 0        | 0 | 0     | 0 |
|                            |                  | 72   |     | 18             | 8      | 10 | 0        | 0 | 0     | 0 |
|                            |                  | 96   |     | 18             | 8      | 10 | 0        | 0 | 0     | 0 |
| 0,32                       | -                | 24   |     | 22             | 10     | 12 | 0        | 0 | 0     | 0 |
|                            |                  | 48   |     | 22             | 10     | 12 | 0        | 0 | 0     | 0 |
|                            |                  | 72   |     | 22             | 9      | 12 | 1        | 0 | 0     | 0 |
|                            |                  | 96   | 7.8 | 22             | 9      | 12 | 1        | 0 | 0     | 0 |
| 0,56                       | -                | 24   |     | 20             | 10     | 9  | 0        | 1 | 0     | 0 |
|                            |                  | 48   |     | 20             | 10     | 5  | 0        | 4 | 0     | 1 |
|                            |                  | 72   |     | 20             | 10     | 0  | 0        | 8 | 0     | 2 |
|                            |                  | 96   |     | 20             | 10     | 0  | 0        | 2 | 0     | 8 |
| 1                          | -                | 24   |     | 20             | 10     | 10 | 0        | 0 | 0     | 0 |
|                            |                  | 48   |     | 20             | 10     | 9  | 0        | 0 | 0     | 1 |
|                            |                  | 72   |     | 20             | 10     | 9  | 0        | 0 | 0     | 1 |
|                            |                  | 96   |     | 20             | 10     | 9  | 0        | 0 | 0     | 1 |
| 1,8                        | -                | 24   | 7.2 | 20             | 10     | 10 | 0        | 0 | 0     | 0 |
|                            |                  | 48   |     | 20             | 10     | 10 | 0        | 0 | 0     | 0 |
|                            |                  | 72   |     | 20             | 10     | 10 | 0        | 0 | 0     | 0 |
|                            |                  | 96   |     | 20             | 10     | 10 | 0        | 0 | 0     | 0 |

| Thiram ( $\mu\text{g/l}$ ) |                  | Time | pH  | n <sup>1</sup> | Mobile |   | Immobile |   | Death |   |
|----------------------------|------------------|------|-----|----------------|--------|---|----------|---|-------|---|
| nom <sup>2</sup>           | act <sup>3</sup> | (hr) |     |                | 1      | 2 | 1        | 2 | 1     | 2 |
| 3,2                        | -                | 24   |     | 18             | 0      | 0 | 10       | 8 | 0     | 0 |
|                            |                  | 48   |     | 18             | 0      | 0 | 8        | 6 | 2     | 2 |
|                            |                  | 72   |     | 18             | 1      | 0 | 3        | 4 | 6     | 4 |
|                            |                  | 96   | 7.8 | 18             | 0      | 0 | 4        | 2 | 6     | 6 |

1: total number of specimens in the duplicates;

2: nominal concentration ( $\mu\text{g/l}$ );

3: actual concentration ( $\mu\text{g/l}$ )

Appendix 8: Influence of Methylisothiocyanate (MITC) (1) on the mortality (%) en mobility (%) of *P. germanica* after 24, 48, 72 and 96 hours of exposure.

Animal capture date: 09-09-91.

Date: 21-10-91. Responsible technician: J.J. Boessenkool.

| MITC ( $\mu\text{g/l}$ ) |                  | Time | pH | n <sup>1</sup> | Mobile |    | Immobile |    | Death |    |
|--------------------------|------------------|------|----|----------------|--------|----|----------|----|-------|----|
| nom <sup>2</sup>         | act <sup>3</sup> | (hr) |    |                | 1      | 2  | 1        | 2  | 1     | 2  |
| 0                        | 0                | 24   |    | 20             | 10     | 10 | 0        | 0  | 0     | 0  |
|                          |                  | 48   |    | 20             | 5      | 9  | 2        | 1  | 3     | 0  |
|                          |                  | 72   |    | 20             | 4      | 6  | 2        | 2  | 4     | 2  |
|                          |                  | 96   |    | 20             | 4      | 6  | 2        | 1  | 4     | 3  |
| 180                      | 160              | 24   |    | 20             | 10     | 10 | 0        | 0  | 0     | 0  |
|                          |                  | 48   |    | 19             | 0      | 0  | 8        | 5  | 1     | 5  |
|                          |                  | 72   |    | 20             | 0      | 0  | 4        | 5  | 6     | 5  |
|                          |                  | 96   |    | 19             | 0      | 0  | 0        | 0  | 10    | 9  |
| 320                      | 360              | 24   |    | 20             | 8      | 7  | 2        | 3  | 0     | 0  |
|                          |                  | 48   |    | 20             | 0      | 0  | 7        | 6  | 3     | 4  |
|                          |                  | 72   |    | 20             | 0      | 0  | 1        | 0  | 9     | 10 |
|                          |                  | 96   |    | 20             | 0      | 0  | 0        | 0  | 10    | 10 |
| 560                      | 520              | 24   |    | 19             | 0      | 0  | 8        | 10 | 1     | 0  |
|                          |                  | 48   |    | 19             | 0      | 0  | 1        | 3  | 8     | 7  |
|                          |                  | 72   |    | 19             | 0      | 0  | 0        | 0  | 9     | 10 |
|                          |                  | 96   |    | 19             | 0      | 0  | 0        | 0  | 9     | 10 |
| 1000                     | 970              | 24   |    | 21             | 0      | 0  | 8        | 8  | 2     | 3  |
|                          |                  | 48   |    | 20             | 0      | 0  | 0        | 0  | 10    | 10 |
|                          |                  | 72   |    | 20             | 0      | 0  | 0        | 0  | 10    | 10 |
|                          |                  | 96   |    | 20             | 0      | 0  | 0        | 0  | 10    | 10 |
| 1800                     | 1700             | 24   |    | 21             | 0      | 0  | 5        | 7  | 5     | 4  |
|                          |                  | 48   |    | 22             | 0      | 0  | 0        | 0  | 11    | 11 |
|                          |                  | 72   |    | 22             | 0      | 0  | 0        | 0  | 11    | 11 |
|                          |                  | 96   |    | 22             | 0      | 0  | 0        | 0  | 11    | 11 |

1: total number of specimens in the duplicates;

2: nominal concentration (mg/l);

3: actual concentration (mg/l)

Appendix 9: Influence of Methylisothiocyanate (MITC) (2) on the mortality (%) en mobility (%) of *P. germanica* after 24, 48, 72 and 96 hours of exposure.

Animal capture date: 13-03-92.

Date: 16-03-92. Responsible technician: J.J. Boessenkool.

| MITC ( $\mu\text{g/l}$ ) |                  | Time | pH | n <sup>1</sup> | Mobile |    | Immobile |    | Death |   |
|--------------------------|------------------|------|----|----------------|--------|----|----------|----|-------|---|
| nom <sup>2</sup>         | act <sup>3</sup> | (hr) |    |                | 1      | 2  | 1        | 2  | 1     | 2 |
| 0                        |                  | 24   |    | 19             | 9      | 10 | 0        | 0  | 0     | 0 |
|                          |                  | 48   |    | 19             | 9      | 10 | 0        | 0  | 0     | 0 |
|                          |                  | 72   |    | 19             | 9      | 10 | 0        | 0  | 0     | 0 |
|                          |                  | 96   |    | 19             | 9      | 10 | 0        | 0  | 0     | 0 |
| 32                       |                  | 24   |    | 20             | 10     | 10 | 0        | 0  | 0     | 0 |
|                          |                  | 48   |    | 20             | 8      | 8  | 2        | 2  | 0     | 0 |
|                          |                  | 72   |    | 20             | 8      | 7  | 2        | 3  | 0     | 0 |
|                          |                  | 96   |    | 20             | 3      | 5  | 6        | 5  | 1     | 0 |
| 56                       |                  | 24   |    | 20             | 8      | 8  | 2        | 2  | 0     | 0 |
|                          |                  | 48   |    | 20             | 3      | 6  | 7        | 4  | 0     | 0 |
|                          |                  | 72   |    | 20             | 2      | 4  | 7        | 6  | 1     | 0 |
|                          |                  | 96   |    | 20             | 0      | 0  | 8        | 10 | 2     | 0 |
| 100                      |                  | 24   |    | 20             | 9      | 8  | 1        | 2  | 0     | 0 |
|                          |                  | 48   |    | 20             | 7      | 7  | 3        | 3  | 0     | 0 |
|                          |                  | 72   |    | 20             | 3      | 2  | 7        | 8  | 0     | 0 |
|                          |                  | 96   |    | 20             | 0      | 0  | 9        | 8  | 1     | 2 |
| 180                      |                  | 24   |    | 20             | 3      | 4  | 7        | 6  | 0     | 0 |
|                          |                  | 48   |    | 20             | 3      | 0  | 7        | 10 | 0     | 0 |
|                          |                  | 72   |    | 20             | 0      | 0  | 9        | 10 | 1     | 0 |
|                          |                  | 96   |    | 20             | 0      | 0  | 4        | 6  | 6     | 4 |
| 320                      |                  | 24   |    | 20             | 2      | 3  | 8        | 7  | 0     | 0 |
|                          |                  | 48   |    | 20             | 0      | 0  | 10       | 10 | 0     | 0 |
|                          |                  | 72   |    | 20             | 0      | 0  | 9        | 7  | 1     | 3 |
|                          |                  | 96   |    | 20             | 0      | 0  | 1        | 1  | 9     | 9 |

1: total number of specimens in the duplicates;

2: nominal concentration (mg/l);

3: actual concentration (mg/l)

Appendix 10: Influence of Methylisothiocyanate (MITC) (3) on the mortality (%) en mobility (%) of *P. germanica* after 24, 48, 72 and 96 hours of exposure.

Animal capture date: 12-11-92

Date: 14-12-92. Responsible technician: J.J. Boessenkool.

| MITC ( $\mu\text{g/l}$ ) |                  | Time | pH  | n <sup>1</sup> | Mobile |    | Immobile |    | Death |    |
|--------------------------|------------------|------|-----|----------------|--------|----|----------|----|-------|----|
| nom <sup>2</sup>         | act <sup>3</sup> | (hr) |     |                | 1      | 2  | 1        | 2  | 1     | 2  |
| 0                        |                  | 24   |     | 20             | 10     | 10 | 0        | 0  | 0     | 0  |
|                          |                  | 48   |     | 20             | 10     | 10 | 0        | 0  | 0     | 0  |
|                          |                  | 72   |     | 20             | 9      | 10 | 1        | 0  | 0     | 0  |
|                          |                  | 96   | 7.4 | 20             | 9      | 10 | 1        | 0  | 0     | 0  |
| 32                       | 27               | 24   |     | 20             | 0      | 0  | 10       | 10 | 0     | 0  |
|                          |                  | 48   |     | 20             | 0      | 0  | 8        | 1  | 2     | 9  |
|                          |                  | 72   |     | 20             | 0      | 0  | 1        | 0  | 9     | 10 |
|                          |                  | 96   | 7.8 | 20             | 0      | 0  | 0        | 0  | 10    | 10 |
| 56                       | 44               | 24   |     | 20             | 7      | 7  | 3        | 3  | 0     | 0  |
|                          |                  | 48   |     | 20             | 7      | 7  | 3        | 3  | 0     | 0  |
|                          |                  | 72   |     | 20             | 2      | 3  | 8        | 7  | 0     | 0  |
|                          |                  | 96   | 7.8 | 20             | 2      | 1  | 7        | 9  | 1     | 0  |
| 100                      | 80               | 24   |     | 20             | 0      | 0  | 6        | 7  | 4     | 3  |
|                          |                  | 48   |     | 20             | 0      | 0  | 0        | 3  | 10    | 7  |
|                          |                  | 72   |     | 20             | 0      | 0  | 0        | 0  | 10    | 10 |
|                          |                  | 96   | 7.8 | 20             | 0      | 0  | 0        | 0  | 10    | 10 |
| 180                      | 145              | 24   |     | 20             | 0      | 0  | 5        | 10 | 5     | 0  |
|                          |                  | 48   |     | 20             | 0      | 0  | 0        | 2  | 10    | 8  |
|                          |                  | 72   |     | 20             | 0      | 0  | 0        | 0  | 10    | 10 |
|                          |                  | 96   | 7.4 | 20             | 0      | 0  | 0        | 0  | 10    | 10 |
| 320                      | 255              | 24   |     | 20             | 0      | 0  | 10       | 10 | 0     | 0  |
|                          |                  | 48   |     | 20             | 0      | 0  | 8        | 0  | 2     | 10 |
|                          |                  | 72   |     | 20             | 0      | 0  | 2        | 0  | 8     | 10 |
|                          |                  | 96   | 7.7 | 20             | 0      | 0  | 2        | 0  | 8     | 10 |
| 560                      | 447              | 24   |     | 19             | 0      | 0  | 3        | 5  | 6     | 5  |
|                          |                  | 48   |     | 19             | 0      | 0  | 0        | 0  | 9     | 10 |
|                          |                  | 72   |     | 19             | 0      | 0  | 0        | 0  | 9     | 10 |
|                          |                  | 96   | 7.7 | 19             | 0      | 0  | 0        | 0  | 9     | 10 |

1: total number of specimens in the duplicates;

2: nominal concentration (mg/l);

3: actual concentration (mg/l)

Appendix 11: Influence of Ethylenethiourea (ETU) on the mortality (%) en mobility (%) of *P. germanica* after 24, 48, 72 and 96 hours of exposure.

Animal capture date: 13-03-92.

Date: 13-04-92. Responsible technician: J.J. Boessenkool.

| ETU (mg/l)       |                  | Time | pH  | n <sup>1</sup> | Mobile |    | Immobile |   | Death |   |
|------------------|------------------|------|-----|----------------|--------|----|----------|---|-------|---|
| nom <sup>2</sup> | act <sup>3</sup> | (hr) |     |                | 1      | 2  | 1        | 2 | 1     | 2 |
| 0                | <0.01            | 24   |     | 20             | 10     | 10 | 0        | 0 | 0     | 0 |
|                  |                  | 48   |     | 20             | 10     | 10 | 0        | 0 | 0     | 0 |
|                  |                  | 72   |     | 20             | 10     | 10 | 0        | 0 | 0     | 0 |
|                  |                  | 96   | 7.7 | 20             | 10     | 10 | 0        | 0 | 0     | 0 |
| 0.1              | 0.1              | 24   |     | 17             | 8      | 9  | 0        | 0 | 0     | 0 |
|                  |                  | 48   |     | 17             | 8      | 9  | 0        | 0 | 0     | 0 |
|                  |                  | 72   |     | 17             | 8      | 8  | 0        | 1 | 0     | 0 |
|                  |                  | 96   |     | 17             | 8      | 8  | 0        | 1 | 0     | 0 |
| 0.18             | 0.18             | 24   |     | 20             | 10     | 10 | 0        | 0 | 0     | 0 |
|                  |                  | 48   |     | 20             | 10     | 9  | 0        | 1 | 0     | 0 |
|                  |                  | 72   |     | 20             | 10     | 8  | 0        | 2 | 0     | 0 |
|                  |                  | 96   | 7.8 | 20             | 9      | 8  | 1        | 2 | 0     | 0 |
| 0.32             | 0.31             | 24   |     | 19             | 9      | 10 | 0        | 0 | 0     | 0 |
|                  |                  | 48   |     | 19             | 9      | 10 | 0        | 0 | 0     | 0 |
|                  |                  | 72   |     | 19             | 7      | 10 | 2        | 0 | 0     | 0 |
|                  |                  | 96   | 7.8 | 19             | 7      | 9  | 2        | 1 | 0     | 0 |
| 0.56             | 0.56             | 24   |     | 17             | 8      | 9  | 0        | 0 | 0     | 0 |
|                  |                  | 48   |     | 18             | 8      | 8  | 0        | 2 | 0     | 0 |
|                  |                  | 72   |     | 18             | 7      | 7  | 1        | 3 | 0     | 0 |
|                  |                  | 96   | 7.6 | 18             | 7      | 6  | 1        | 3 | 0     | 1 |
| 1.0              | 0.98             | 24   |     | 20             | 9      | 8  | 1        | 2 | 0     | 0 |
|                  |                  | 48   |     | 20             | 9      | 8  | 0        | 2 | 1     | 0 |
|                  |                  | 72   |     | 20             | 9      | 7  | 0        | 3 | 1     | 0 |
|                  |                  | 96   | 7.6 | 20             | 7      | 6  | 2        | 4 | 1     | 0 |

1: total number of specimens in the duplicates;

2: nominal concentration (mg/l);

3: actual concentration (mg/l).

Appendix 12: Influence of Cypermethrin (1) on the mortality (%) en mobility (%) of *P. germanica* after 24, 48, 72 and 96 hours of exposure.

Animal capture date: 13-03-92.

Date: 13-04-92. Responsible technician: J.J. Boessenkool.

| Cypermethrin ( $\mu\text{g/l}$ ) |                   | Time | n <sup>1</sup> | pH  | Mobile |    | Immobile |    | Death |   |
|----------------------------------|-------------------|------|----------------|-----|--------|----|----------|----|-------|---|
| nom <sup>2</sup>                 | actu <sup>3</sup> | (hr) |                |     | 1      | 2  | 1        | 2  | 1     | 2 |
| 0                                |                   | 24   | 20             |     | 10     | 10 | 0        | 0  | 0     | 0 |
|                                  |                   | 48   | 20             |     | 10     | 10 | 0        | 0  | 0     | 0 |
|                                  |                   | 72   | 20             |     | 10     | 10 | 0        | 0  | 0     | 0 |
|                                  |                   | 96   | 20             |     | 10     | 10 | 0        | 0  | 0     | 0 |
| 0 <sup>4</sup>                   |                   | 24   | 20             |     | 10     | 10 | 0        | 0  | 0     | 0 |
|                                  |                   | 48   | 20             |     | 10     | 10 | 0        | 0  | 0     | 0 |
|                                  |                   | 72   | 20             |     | 10     | 10 | 0        | 0  | 0     | 0 |
|                                  |                   | 96   | 20             | 7.7 | 10     | 10 | 0        | 0  | 0     | 0 |
| 0.1                              |                   | 24   | 20             |     | 0      | 0  | 10       | 10 | 0     | 0 |
|                                  |                   | 48   | 20             |     | 0      | 0  | 9        | 9  | 1     | 1 |
|                                  |                   | 72   | 20             |     | 0      | 0  | 6        | 8  | 4     | 2 |
|                                  |                   | 96   | 20             |     | 0      | 0  | 5        | 8  | 5     | 2 |
| 0.18                             |                   | 24   | 20             |     | 0      | 0  | 10       | 10 | 0     | 0 |
|                                  |                   | 48   | 20             |     | 0      | 0  | 10       | 10 | 0     | 0 |
|                                  |                   | 72   | 20             |     | 0      | 0  | 10       | 10 | 0     | 0 |
|                                  |                   | 96   | 20             | 8.0 | 0      | 0  | 8        | 7  | 2     | 3 |
| 0.32                             |                   | 24   | 20             |     | 0      | 0  | 9        | 10 | 1     | 0 |
|                                  |                   | 48   | 20             |     | 0      | 0  | 8        | 9  | 2     | 1 |
|                                  |                   | 72   | 20             |     | 0      | 0  | 10       | 8  | 0     | 2 |
|                                  |                   | 96   | 20             | 7.8 | 0      | 0  | 6        | 7  | 4     | 3 |
| 0.56                             |                   | 24   | 20             |     | 0      | 0  | 9        | 9  | 1     | 1 |
|                                  |                   | 48   | 20             |     | 0      | 0  | 8        | 8  | 2     | 2 |
|                                  |                   | 72   | 20             |     | 0      | 0  | 8        | 5  | 2     | 5 |
|                                  |                   | 96   | 20             |     | 0      | 0  | 4        | 4  | 6     | 6 |
| 1.0                              |                   | 24   | 19             |     | 0      | 0  | 7        | 3  | 2     | 7 |
|                                  |                   | 48   | 19             |     | 0      | 0  | 6        | 2  | 3     | 8 |
|                                  |                   | 72   | 19             |     | 0      | 0  | 4        | 2  | 6     | 8 |
|                                  |                   | 96   | 19             | 7.9 | 0      | 0  | 4        | 2  | 6     | 8 |

1: total number of specimens in the duplicates;

2: nominal concentration (mg/l);

3: actual concentration (mg/l);

4: solvent acetone, 0.001% in 0<sup>+</sup>.

Appendix 13: Influence of Cypermethrin (2) on the mortality (%) en mobility (%) of *P. germanica* after 24, 48, 72 and 96 hours of exposure.

Animal capture date: 15-04-93.

Date: 26-04-93. Responsible technician: J.J. Boessenkool.

| Cypermethrin ( $\mu\text{g/l}$ ) |                  | Time | n <sup>1</sup> | pH  | Mobile |    | Immobile |    | Death |   |
|----------------------------------|------------------|------|----------------|-----|--------|----|----------|----|-------|---|
| nom <sup>2</sup>                 | act <sup>3</sup> | (hr) |                |     | 1      | 2  | 1        | 2  | 1     | 2 |
| 0                                |                  | 24   | 20             |     | 10     | 10 | 0        | 0  | 0     | 0 |
|                                  |                  | 48   | 20             |     | 10     | 10 | 0        | 0  | 0     | 0 |
|                                  |                  | 72   | 20             |     | 10     | 10 | 0        | 0  | 0     | 0 |
|                                  |                  | 96   | 20             | 7.3 | 10     | 10 | 0        | 0  | 0     | 0 |
| 0 <sup>++</sup>                  |                  | 24   | 20             |     | 10     | 10 | 0        | 0  | 0     | 0 |
|                                  |                  | 48   | 20             |     | 10     | 10 | 0        | 0  | 0     | 0 |
|                                  |                  | 72   | 20             |     | 10     | 10 | 0        | 0  | 0     | 0 |
|                                  |                  | 96   | 20             | 7.7 | 10     | 10 | 0        | 0  | 0     | 0 |
| 0.01                             |                  | 24   | 20             |     | 10     | 10 | 0        | 0  | 0     | 0 |
|                                  |                  | 48   | 20             |     | 10     | 10 | 0        | 0  | 0     | 0 |
|                                  |                  | 72   | 20             |     | 9      | 10 | 1        | 0  | 0     | 0 |
|                                  |                  | 96   | 20             |     | 9      | 10 | 1        | 0  | 0     | 0 |
| 0.032                            |                  | 24   | 20             |     | 9      | 7  | 1        | 3  | 0     | 0 |
|                                  |                  | 48   | 20             |     | 6      | 2  | 4        | 8  | 0     | 0 |
|                                  |                  | 72   | 20             |     | 2      | 0  | 8        | 9  | 0     | 1 |
|                                  |                  | 96   | 20             | 7.8 | 2      | 0  | 8        | 9  | 0     | 1 |
| 0.056                            |                  | 24   | 21             |     | 1      | 2  | 9        | 9  | 0     | 0 |
|                                  |                  | 48   | 21             |     | 0      | 0  | 10       | 11 | 0     | 0 |
|                                  |                  | 72   | 21             |     | 0      | 0  | 10       | 11 | 0     | 0 |
|                                  |                  | 96   | 21             | 7.8 | 0      | 0  | 10       | 11 | 0     | 0 |
| 0.1                              |                  | 24   | 20             |     | 2      | 1  | 8        | 9  | 0     | 0 |
|                                  |                  | 48   | 20             |     | 0      | 0  | 10       | 10 | 0     | 0 |
|                                  |                  | 72   | 20             |     | 0      | 0  | 10       | 10 | 0     | 0 |
|                                  |                  | 96   | 20             |     | 0      | 0  | 10       | 10 | 0     | 0 |
| 0.32                             |                  | 24   | 20             |     | 0      | 0  | 10       | 9  | 0     | 1 |
|                                  |                  | 48   | 20             |     | 0      | 0  | 10       | 9  | 0     | 1 |
|                                  |                  | 72   | 19             |     | 0      | 0  | 8        | 8  | 2     | 1 |
|                                  |                  | 96   | 19             | 7.5 | 0      | 0  | 7        | 7  | 3     | 2 |
| 0.56                             |                  | 24   | 19             |     | 0      | 0  | 9        | 10 | 0     | 0 |
|                                  |                  | 48   | 19             |     | 0      | 0  | 9        | 10 | 0     | 0 |
|                                  |                  | 72   | 19             |     | 0      | 0  | 8        | 9  | 1     | 1 |
|                                  |                  | 96   | 19             |     | 0      | 0  | 6        | 9  | 3     | 1 |

| Cypermethrin ( $\mu\text{g/l}$ ) |                  | Time | n <sup>1</sup> | pH  | Mobile |   | Immobile |    | Death |   |
|----------------------------------|------------------|------|----------------|-----|--------|---|----------|----|-------|---|
| nom <sup>2</sup>                 | act <sup>3</sup> | (hr) |                |     | 1      | 2 | 1        | 2  | 1     | 2 |
| 1.0                              |                  | 24   | 19             |     | 0      | 0 | 9        | 10 | 0     | 0 |
|                                  |                  | 48   | 19             |     | 0      | 0 | 9        | 10 | 0     | 0 |
|                                  |                  | 72   | 19             |     | 0      | 0 | 7        | 7  | 2     | 3 |
|                                  |                  | 96   | 19             | 7.4 | 0      | 0 | 5        | 5  | 4     | 5 |
| 3.2                              |                  | 24   | 20             |     | 0      | 0 | 9        | 10 | 1     | 0 |
|                                  |                  | 48   | 20             |     | 0      | 0 | 9        | 10 | 1     | 0 |
|                                  |                  | 72   | 20             |     | 0      | 0 | 7        | 6  | 3     | 4 |
|                                  |                  | 96   | 20             |     | 0      | 0 | 4        | 5  | 6     | 5 |
| 5.6                              |                  | 24   | 20             |     | 0      | 0 | 9        | 10 | 1     | 0 |
|                                  |                  | 48   | 20             |     | 0      | 0 | 9        | 9  | 1     | 1 |
|                                  |                  | 72   | 20             |     | 0      | 0 | 4        | 6  | 6     | 4 |
|                                  |                  | 96   | 20             | 7.9 | 0      | 0 | 2        | 3  | 8     | 7 |

1: total number of specimens in the duplicates;

2: nominal concentration (mg/l);

3: actual concentration (mg/l);

4: solvent ethylacetate, 0.001% in 0<sup>+</sup>.

Appendix 14: Influence of Ethoprophos on the mortality (%) en mobility (%) of *P. germanica* after 24, 48, 72 and 96 hours of exposure.

Animal capture date: 15-04-93.

Date: 19-04-93. Responsible technician: J.J. Boessenkool.

| Ethoprophos ( $\mu\text{g/l}$ ) |                   | Time | n <sup>1</sup> | pH  | Mobile |    | Immobile |   | Death |   |
|---------------------------------|-------------------|------|----------------|-----|--------|----|----------|---|-------|---|
| nom <sup>2</sup>                | actu <sup>3</sup> | (hr) |                |     | 1      | 2  | 1        | 2 | 1     | 2 |
| 0                               |                   | 24   | 17             |     | 8      | 8  | 0        | 1 | 0     | 0 |
|                                 |                   | 48   | 17             |     | 8      | 8  | 0        | 1 | 0     | 0 |
|                                 |                   | 72   | 17             |     | 8      | 8  | 0        | 0 | 0     | 1 |
|                                 |                   | 96   | 17             | 7.5 | 8      | 8  | 0        | 0 | 0     | 1 |
| 0 <sup>++</sup>                 |                   | 24   | 20             |     | 10     | 10 | 0        | 0 | 0     | 0 |
|                                 |                   | 48   | 20             |     | 10     | 10 | 0        | 0 | 0     | 0 |
|                                 |                   | 72   | 20             |     | 10     | 10 | 0        | 0 | 0     | 0 |
|                                 |                   | 96   | 20             | 7.7 | 10     | 10 | 0        | 0 | 0     | 0 |
| 3.2                             | <0.1              | 24   | 20             |     | 10     | 10 | 0        | 0 | 0     | 0 |
|                                 |                   | 48   | 20             |     | 10     | 10 | 0        | 0 | 0     | 0 |
|                                 |                   | 72   | 20             |     | 10     | 10 | 0        | 0 | 0     | 0 |
|                                 |                   | 96   | 20             | 7.8 | 10     | 10 | 0        | 0 | 0     | 0 |
| 5.6                             | <0.1              | 24   | 19             |     | 9      | 10 | 0        | 0 | 0     | 0 |
|                                 |                   | 48   | 19             |     | 9      | 10 | 0        | 0 | 0     | 0 |
|                                 |                   | 72   | 19             |     | 9      | 10 | 0        | 0 | 0     | 0 |
|                                 |                   | 96   | 19             | 7.7 | 8      | 10 | 1        | 0 | 0     | 0 |
| 10                              | 2.6               | 24   | 20             |     | 10     | 10 | 0        | 0 | 0     | 0 |
|                                 |                   | 48   | 20             |     | 10     | 10 | 0        | 0 | 0     | 0 |
|                                 |                   | 72   | 20             |     | 9      | 8  | 1        | 2 | 0     | 0 |
|                                 |                   | 96   | 20             | 7.6 | 9      | 8  | 0        | 2 | 1     | 0 |
| 32                              | 26.4              | 24   | 21             |     | 10     | 9  | 0        | 2 | 0     | 0 |
|                                 |                   | 48   | 21             |     | 9      | 9  | 1        | 1 | 0     | 1 |
|                                 |                   | 72   | 21             |     | 9      | 9  | 0        | 1 | 1     | 1 |
|                                 |                   | 96   | 21             | 7.7 | 9      | 9  | 0        | 0 | 1     | 2 |
| 56                              | 26.4              | 24   | 20             |     | 10     | 10 | 0        | 0 | 0     | 0 |
|                                 |                   | 48   | 20             |     | 9      | 10 | 1        | 0 | 0     | 0 |
|                                 |                   | 72   | 20             |     | 9      | 10 | 1        | 0 | 0     | 0 |
|                                 |                   | 96   | 20             | 7.4 | 9      | 10 | 0        | 0 | 1     | 0 |

| Ethoprosfos ( $\mu\text{g/l}$ ) |                   | Time | n <sup>1</sup> | pH  | Mobile |    | Immobile |   | Death |   |
|---------------------------------|-------------------|------|----------------|-----|--------|----|----------|---|-------|---|
| nom <sup>2</sup>                | actu <sup>3</sup> | (hr) |                |     | 1      | 2  | 1        | 2 | 1     | 2 |
| 100                             | 111               | 24   | 19             |     | 10     | 9  | 0        | 0 | 0     | 0 |
|                                 |                   | 48   | 19             |     | 10     | 9  | 0        | 0 | 0     | 0 |
|                                 |                   | 72   | 19             |     | 10     | 8  | 0        | 1 | 0     | 0 |
|                                 |                   | 96   | 19             | 7.6 | 10     | 8  | 0        | 1 | 0     | 0 |
| 320                             | 304               | 24   | 19             |     | 9      | 10 | 0        | 0 | 0     | 0 |
|                                 |                   | 48   | 19             |     | 9      | 10 | 0        | 0 | 0     | 0 |
|                                 |                   | 72   | 19             |     | 8      | 9  | 1        | 1 | 0     | 0 |
|                                 |                   | 96   | 19             | 7.7 | 8      | 9  | 0        | 0 | 1     | 1 |
| 560                             | 581               | 24   | 18             |     | 9      | 8  | 0        | 0 | 0     | 1 |
|                                 |                   | 48   | 18             |     | 5      | 4  | 4        | 4 | 0     | 1 |
|                                 |                   | 72   | 18             |     | 1      | 2  | 5        | 6 | 3     | 1 |
|                                 |                   | 96   | 18             | 7.8 | 1      | 1  | 4        | 4 | 4     | 4 |
| 1000                            | 1082              | 24   | 19             |     | 2      | 2  | 5        | 4 | 3     | 3 |
|                                 |                   | 48   | 19             |     | 2      | 0  | 5        | 7 | 3     | 2 |
|                                 |                   | 72   | 19             |     | 0      | 0  | 6        | 3 | 4     | 6 |
|                                 |                   | 96   | 19             | 7.6 | 0      | 0  | 0        | 0 | 10    | 9 |

1: total number of specimens in the duplicates;

2: nominal concentration (mg/l);

3: actual concentration (mg/l);

4: solvent ethylacetate, 0.001% in O<sup>+</sup>.

Appendix 15: Influence of Fenpropathrin on the mortality (%) en mobility (%) of *P. germanica* after 24, 48, 72 and 96 hours of exposure.

Animal capture date: .....93.

Date: 10-05-93. Responsible technician: J.J. Boessenkool.

| Fenpropathrin ( $\mu\text{g/l}$ ) |                   | Time | n <sup>1</sup> | pH  | Mobile |    | Immobile |    | Death |   |
|-----------------------------------|-------------------|------|----------------|-----|--------|----|----------|----|-------|---|
| nom <sup>2</sup>                  | actu <sup>3</sup> | (hr) |                |     | 1      | 2  | 1        | 2  | 1     | 2 |
| 0                                 |                   | 24   | 20             |     | 10     | 10 | 0        | 0  | 0     | 0 |
|                                   |                   | 48   | 20             |     | 10     | 10 | 0        | 0  | 0     | 0 |
|                                   |                   | 72   | 20             |     | 10     | 10 | 0        | 0  | 0     | 0 |
|                                   |                   | 96   | 20             | 7.3 | 10     | 10 | 0        | 0  | 0     | 0 |
| 0 <sup>+</sup>                    |                   | 24   | 20             |     | 10     | 10 | 0        | 0  | 0     | 0 |
|                                   |                   | 48   | 20             |     | 10     | 10 | 0        | 0  | 0     | 0 |
|                                   |                   | 72   | 20             |     | 10     | 10 | 0        | 0  | 0     | 0 |
|                                   |                   | 96   | 20             | 7.7 | 10     | 10 | 0        | 0  | 0     | 0 |
| 0.0032                            | s                 | 24   | 21             |     | 4      | 6  | 7        | 4  | 0     | 0 |
|                                   |                   | 48   | 21             |     | 4      | 6  | 7        | 4  | 0     | 0 |
|                                   |                   | 72   | 21             |     | 4      | 6  | 6        | 4  | 1     | 0 |
|                                   |                   | 96   | 21             |     | 4      | 6  | 6        | 4  | 1     | 0 |
| 0.01                              | s                 | 24   | 20             |     | 9      | 7  | 1        | 3  | 0     | 0 |
|                                   |                   | 48   | 20             |     | 6      | 2  | 4        | 8  | 0     | 0 |
|                                   |                   | 72   | 20             |     | 2      | 0  | 8        | 9  | 0     | 1 |
|                                   |                   | 96   | 20             | 7.8 | 2      | 0  | 8        | 9  | 0     | 1 |
| 0.032                             | s                 | 24   | 21             |     | 1      | 2  | 9        | 9  | 0     | 0 |
|                                   |                   | 48   | 21             |     | 0      | 0  | 10       | 11 | 0     | 0 |
|                                   |                   | 72   | 21             |     | 0      | 0  | 10       | 11 | 0     | 0 |
|                                   |                   | 96   | 21             | 7.8 | 0      | 0  | 10       | 11 | 0     | 0 |
| 0.1                               | <0.005            | 24   | 20             |     | 2      | 1  | 8        | 9  | 0     | 0 |
|                                   |                   | 48   | 20             |     | 0      | 0  | 9        | 10 | 1     | 0 |
|                                   |                   | 72   | 20             |     | 0      | 0  | 10       | 10 | 0     | 0 |
|                                   |                   | 96   | 20             |     | 0      | 0  | 10       | 10 | 0     | 0 |
| 0.32                              | 0.54              | 24   | 20             |     | 0      | 0  | 10       | 9  | 0     | 1 |
|                                   |                   | 48   | 20             |     | 0      | 0  | 10       | 9  | 0     | 1 |
|                                   |                   | 72   | 20             |     | 0      | 0  | 8        | 8  | 2     | 1 |
|                                   |                   | 96   | 20             | 7.5 | 0      | 0  | 7        | 7  | 3     | 2 |
| 1.0                               | 0.44              | 24   | 19             |     | 0      | 0  | 9        | 10 | 0     | 0 |
|                                   |                   | 48   | 19             |     | 0      | 0  | 9        | 10 | 0     | 0 |
|                                   |                   | 72   | 19             |     | 0      | 0  | 8        | 9  | 1     | 1 |
|                                   |                   | 96   | 19             |     | 0      | 0  | 6        | 9  | 3     | 1 |
| 3.2                               | 0.49              | 24   | 19             |     | 0      | 0  | 10       | 9  | 0     | 0 |

| Fenpropathrin ( $\mu\text{g/l}$ ) |                   | Time | n <sup>1</sup> | pH  | Mobile |   | Immobile |   | Death |    |
|-----------------------------------|-------------------|------|----------------|-----|--------|---|----------|---|-------|----|
| nom <sup>2</sup>                  | actu <sup>3</sup> | (hr) |                |     | 1      | 2 | 1        | 2 | 1     | 2  |
|                                   |                   | 48   | 19             |     | 0      | 0 | 7        | 5 | 3     | 4  |
|                                   |                   | 72   | 19             |     | 0      | 0 | 5        | 5 | 5     | 4  |
|                                   |                   | 96   | 19             | 7.4 | 0      | 0 | 5        | 4 | 5     | 5  |
| 10.0                              | 4.4               | 24   | 19             |     | 0      | 0 | 6        | 6 | 3     | 4  |
|                                   |                   | 48   | 19             |     | 0      | 0 | 2        | 3 | 7     | 7  |
|                                   |                   | 72   | 19             |     | 0      | 0 | 2        | 1 | 7     | 9  |
|                                   |                   | 96   | 19             |     | 0      | 0 | 0        | 1 | 9     | 9  |
| 32                                | 10.1              | 24   | 20             |     | 0      | 0 | 0        | 1 | 10    | 9  |
|                                   |                   | 48   | 20             |     | 0      | 0 | 0        | 0 | 10    | 10 |
|                                   |                   | 72   | 20             |     | 0      | 0 | 0        | 0 | 10    | 10 |
|                                   |                   | 96   | 20             |     | 0      | 0 | 0        | 0 | 10    | 10 |
| 100                               | 41.4              | 24   | 20             |     | 0      | 0 | 0        | 0 | 10    | 10 |
|                                   |                   | 48   | 20             |     | 0      | 0 | 0        | 0 | 10    | 10 |
|                                   |                   | 72   | 20             |     | 0      | 0 | 0        | 0 | 10    | 10 |
|                                   |                   | 96   | 20             | 7.9 | 0      | 0 | 0        | 0 | 10    | 10 |

- 1: total number of specimens in the duplicates;  
2: nominal concentration (mg/l);  
3: actual concentration (mg/l);  
4: solvent ethylacetate, 0.001% in 0<sup>+</sup>;  
5: too few substance available.